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PATENT
38-21(404)A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re The Application Of:	)	
ROBERT T. FRALEY and STEPHEN G. ROGERS	)	Group Art Unit: 184
Serial Number: 931,492	)	Examiner: DAVID T. FOX
Filed: NOVEMBER 17, 1986	)	DECEMBER 12, 1989
Title: CHIMERIC GENES FOR TRANSFORMING PLANT CELLS USING VIRAL PROMOTERS	)	

Considered 3/15/90 DTF

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Stephen G. Rogers, declare that I am a United States citizen and a resident of St. Louis County, Missouri; that I am a research biologist by profession, having been graduated by the Johns Hopkins University, with a degree of Doctor of Philosophy in Biology in 1976. I further declare that since 1980 I have been employed by Monsanto Company in plant molecular biology research and that my present title is Fellow.

I further declare that I am knowledgeable of the scientific literature in the field of molecular biology and that I am one of the inventors of the instant application (Serial No. 931,492). In view of the teachings of the instant application and the available skill in the art as of the time of the making of the invention of this application, one skilled in the art would not have known or expected that a chimeric gene containing a promoter region from a plant virus and a heterologous gene would have been highly expressed in plant cells.

At the time the instant invention was made, it was not known whether the use of an isolated plant virus promoter (such as the CaMV35S or CaMV19S) would enable one to obtain expression of a heterologous gene in a transgenic plant. A number of factors could have influenced the ability of the CaMV promoter to function properly in a plant cell. One such potential problem was that a trans-acting protein from the CaMV DNA may have been required to stimulate or cause activity of the CaMV promoter. If the isolated fragment from CaMV

containing the putative promoter did not also produce the trans-acting protein, the promoter would be inactive or only active at a much reduced level. This possibility was certainly reasonable in view of studies on bacteriophages and mammalian viruses which showed that other proteins were essential for transcription of some genes.

Studies of coliphages T4 and T7 showed that these bacterial viruses produce proteins that are absolutely essential for the transcription of virus late genes (Luria et al., General Virology 3rd edition 1978). The viral encoded proteins are essential for transcription, and in the case of T7, the essential virus protein is an RNA polymerase that can recognize and use promoter sequences in the virus DNA (Studier, F.W., Science 176,367-376, 1972). In the case of the T4 phage, a new protein accessory factor (called a sigma factor) that modifies the specificity of the host RNA polymerase is synthesized (Burgess, R. and Travers, A.A., Nature 222, 537-540, 1969). The accessory protein allows the host polymerase to recognize and initiate mRNA synthesis at virus promoter sequences.

Virus encoded proteins have subsequently been shown to be required for the expression of promoters and production of RNAs in mammalian viruses. The early and late genes of Herpes Simplex Virus require a class of proteins encoded by the virus, called E1 proteins, for their transcription (Clements, J.B. et al., Cell, Vol. 12, 275-295, 1977). The E1 types of proteins are made and required by many different classes of viruses including HTLV (Green and Lowenstein, Cell, Vol.55, 1179-1188, 1988; Chen, Cell, Vol. 47, 1-2, 1986). If the activator protein is not made, the level of subsequent RNA synthesis is much reduced.

The data from bacterial viruses and animal viruses suggest that the high level of transcription seen during infection of plants by CaMV might not be seen with chimeric gene constructs because of a lack of a potentially required virus protein factor for optimal transcription. At the time the instant invention was made, there was not enough information about the biology of CaMV to permit predictions as to whether such a protein factor was made by CaMV or necessary for the transcription of its genes.

Furthermore, it became known at least as early as November of 1983 that in order to obtain expression of heterologous genes in plants, transcriptional signals from a gene that is known to be functional in plants would need to be used "Introduction of Genetic Material into Plant Cells" by A. Caplan et al., Science Vol.222. 18 Nov. 1983, pp.815-821. In the Caplan et al. article, on p.818, Caplan et al. describe attempts to introduce foreign DNA into plant cells which were

successful, but failed to obtain expression of any of these introduced DNAs. Caplan et al. show that both prokaryotic and non-plant eukaryotic genes failed to be expressed in plant cells, even though they may have been physically introduced into the cells. In particular, Caplan et al. note that genes from mammalian cells were not transcribed in plant cells. Therefore, teachings from references attempting to use mammalian genes (including mammalian promoters) could not be used by those skilled in the art to teach what would work or what would be necessary to obtain expression of a heterologous gene in plant cells. Thus, the teachings of the Anderson patent (No. 4,536,475) is incapable of obtaining plant cells that express a heterologous gene since it is only shown to be coupled with a mammalian promoter from the Herpes Simplex Virus thymidine kinase gene.

In summary, I declare that at the time the instant invention was made those skilled in the art would not have known whether a chimeric gene including a promoter from the CaMV would have been capable of obtaining expression of the gene of interest at a noticeable level because of all of the unknown factors involved in the use of a chimeric gene. As other researchers had shown, trans-acting proteins or other factors were necessary for the expression and production of bacteriophage genes. A class of proteins have also been shown to be necessary for the expression of some of the genes of mammalian viruses such as Herpes Simplex Virus. Other factors such as host specificity or structural requirements may also have been involved. From merely knowing that the genes driven by the CaMV promoter in the CaMV itself were expressed at a high level did not lend itself to the conclusion that the use of the promoter sequence in isolation of the rest of the CaMV genome would be capable of obtaining high levels of expression of a heterologous gene in a different host.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Stephen G Rogers

Stephen G. Rogers, Ph.D.

Date Dec 12 1989